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14. ABSTRACT

Repetitive mild TBI leads to chronic traumatic encephalopathy (CTE), but the underlying molecular changes remain unclear. Here, biochemical and genetic studies that deepen our understanding of the pathogenesis of CTE will be performed, facilitating diagnosis and therapeutic development.

15. SUBJECT TERMS

Tau, genetics, susceptibility, MAPT, chronic traumatic encephalopathy, Alzheimer disease

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INTRODUCTION:

Repetitive mild traumatic brain injury leads to neurological symptoms and chronic traumatic encephalopathy (CTE). The molecular changes underlying CTE are unknown, but our data demonstrate a spectrum of pathology with accumulation of aggregates of the microtubule-associated protein tau. Our preliminary data indicates an association between CTE and the tau gene (*MAPT*) H1 haplotype. How *MAPT* haplotypes contribute to CTE is unclear, but differences in transcription, mRNA splicing and translation may participate. The objective of this proposal is to validate the association of CTE with MAPT and characterize differences the expression of tau protein and tau-associated proteins in CTE patients. We hypothesize that the tau H1 haplotype increases CTE risk by increasing expression of abnormal tau. We will test this hypothesis by performing biochemical and genetic studies of CTE. Understanding the mechanisms that underlie changes in tau in CTE will enable biomarkers and treatments. These studies will deepen our understanding of how genetic and biochemical alterations in tau contribute to CTE, facilitating diagnosis and therapeutic development.

KEYWORDS:

Chronic traumatic encephalopathy, tauopathy, tau haplotype, MAPT, tau isoform

ACCOMPLISHMENTS:

What were the major goals of the project?

Our *long-term goal* is to identify molecular mechanisms regulating tau that can be used as diagnostics and to develop therapeutics for CTE. The *immediate goal* of this proposal is to correlate neuropathological findings with *MAPT* variation and discover differences in tau and tau-associated proteins in post-mortem brain, CSF and serum/plasma from CTE that will serve as potential biomarkers and facilitate future drug trials. We *hypothesize* that the neuropathological findings we are currently characterizing in individuals with CTE reflect molecular and genetic differences that will enable the development of biomarkers and therapeutics. To achieve these goals, we pursued the following specific aims:

Aim 1. To discover molecular signatures of CTE stage and severity.

Aim 2. To discover and validate candidate risk alleles for CTE.

Table 1. Major tasks from the statement of work

Specific Aim 1(specified in proposal)	**Timeline**	**Site 1**	**Site 2**	**Status**
Major Task 1: Histopathological correlates of CTE severity and progression	Months			
Subtask 1: Review of autopsy material, case selection, tissue retrieval	1-3	Dr. Crary	Dr. McKee	**Complete**
Subtask 2: Tissue embedding, sectioning and screening by histopathology	4-6	Dr. Crary	Dr. McKee	**Complete**
Subtask 3: Evaluation of neuronal, glial and inflammatory cellular changes	7-12	Dr. Crary	Dr. McKee	Ongoing
Milestone(s) Achieved: Completion of histopathological analysis	12			
Major Task 2. Tau isoform analysis				
Subtask 1. Review of autopsy material, case selection, tissue retrieval	25-28	Dr. Crary	Dr. McKee	**Complete**
Subtask 2. Tissue embedding, sectioning and screening by histopathology	29-31	Dr. Crary	Dr. McKee	Ongoing
Subtask 3. Tau immunoblots, ELISAs and EM	31-36	Dr. Crary		
Milestone(s) Achieved: Completion of biochemical analysis	36			
Specific Aim 2				
Major Task 3. To discover risk alleles for CTE				
Subtask 1. Review of autopsy material, case selection and DNA isolation	13-18	Dr. Crary	Dr. McKee	**Complete**
Subtask 2. MAPT haplotype analysis	19-21	Dr. Crary		**Complete**
Subtask 3. MAPT resequencing	22-24	Dr. Crary		Ongoing
Milestone(s) Achieved: Completion of MAPT haplotype analysis	24			

What was accomplished under these goals?

Note: *This award was in the transfer process from Columbia University Medical Center to the Icahn School of Medicine at Mount Sinai. As such, no funds were available to conduct work until the transfer, effective on October 28th, 2015.*

The major activities in the third year of this project related to the continued histopathological analyses of post-mortem brain tissues and the completion of the genetic studies of *MAPT* haplotypes. As stated in the previous report, we prioritized the haplotype analysis given the high interest in the genetics of CTE. The biochemical analysis of tau isoforms will be conducted in the final year of the award.

1. **Case material was reviewed, and tissue was selected for genotyping, and DNA isolated (Task 3, subtask 1) and initial ancestry analysis and *MAPT* haplotype analysis was performed (Task 3, subtask 2).** DNA was isolated from the brains of subjects with neuropathologically confirmed CTE. The *MAPT* haplotype was determined using haplotype tagging SNPs. Haplotype and allele frequencies were compared to population, autopsy and athlete controls.

Introduction

Our goal is to uncover common variation in *MAPT* and determine whether it is associated with severity of CTE. Our previous first series of association analyses reported last year began to compare autopsy confirmed CTE patients (*n*=103) with a series of control groups with publically available genotype data. The *MAPT* haplotype allele frequency varies considerably among populations, and this can lead to hidden sample stratification. We performed an ancestry analysis using a minimal series of markers with modifications (Phillips et al., 2012). We uncovered good separation of European ancestry from African and Asian reference populations obtains from the 1000 genomes project reference panel. Targeted genotyping was performed on the Sequenom iPlex Massarray platform and statistical analyses in plink. However, this analysis proved insufficient to resolve differences in European sub-populations, a critical confounding variable (*see 2016 annual report*), and more extensive genotyping of ancestry markers was undertaken.

Determination of population structure

This year, we expanded our genotyping to a total of 312 autopsy-confirmed athletes and veterans, all with exposure to mild-repetitive TBI (Table 1). 191 of these subjects had high-quality DNA isolated from fresh-frozen tissue, and the remaining 122, from which the DNA was isolated from formalin-fixed paraffin embedded tissue, the DNA required restoration using an DNA restore kit. Genotyping was performed using an Infinium Omni-Express-24 v1.2 BeadChip (Illumina, San Diego, CA) with a total 713,599 SNPs assessed. The quality control and further association analyses were conducted using PLINK. Sex was confirmed by both counting genotypes on Y-chromosome and computing the inbreeding coefficient (F). Cases with discrepant sex calls and over 5% missing SNP calls were excluded. Additional, individual SNPs with over 5% missing calls were also filtered. Our final cohort was, thus, comprised of 151 unrelated males with 574,139 variants genotyped (Table 1). We used IMPUTE2 for imputation using the cleaned data against the 1000 Genomes Phase 3 reference. After imputation, only SNPs with an info value >= 0.7 were retained.

Table 1. Patient data*

		Age of death	Age of 1st exposure	Years of exposure	Age of 1st Symptoms
CTE stage	***n***	**Mean ± Stdev (range)**	**Mean ± Stdev (range)**	**Mean ± Stdev (range)**	**Mean ± Stdev (range)**
0	39	50.2 ± 26.2 (14-88)	11.3 ± 5 (5-24)	10.3 ± 8.6 (0-38)	NA ± NA (NA)
I	18	53.2 ± 23.7 (22-89)	11.1 ± 2.9 (6-15)	13.9 ± 11.6 (5-58)	40.7 ± 19.4 (17-74)
II	27	51.6 ± 18.8 (24-89)	10.4 ± 3.6 (3-16)	15.9 ± 5.6 (8-35)	37.6 ± 17.3 (17-79)
III	31	64.1 ± 15.3 (25-89)	11.3 ± 2.5 (5-14)	17 ± 4.3 (8-25)	49.1 ± 16.7 (21-83.5)
IV	36	76.6 ± 9.2 (60-98)	12.9 ± 2.6 (6-18)	15.9 ± 7.1 (4-47)	55.2 ± 17.7 (20-83)
Total	151	59.8 ± 21.9 (14-98)	11.5 ± 3.5 (3-24)	14.7 ± 7.8 (0-58)	44.8 ± 20.4 (5-83)

* In years, mean ± standard deviation (range)

We assessed the population structure using two approaches. We performed a principle component analysis (pyGenClean) and found that our cohort could be divided into two major groups. One group consisted of a roughly European cohort with varying degrees of admixture centered near and encompassing the reference

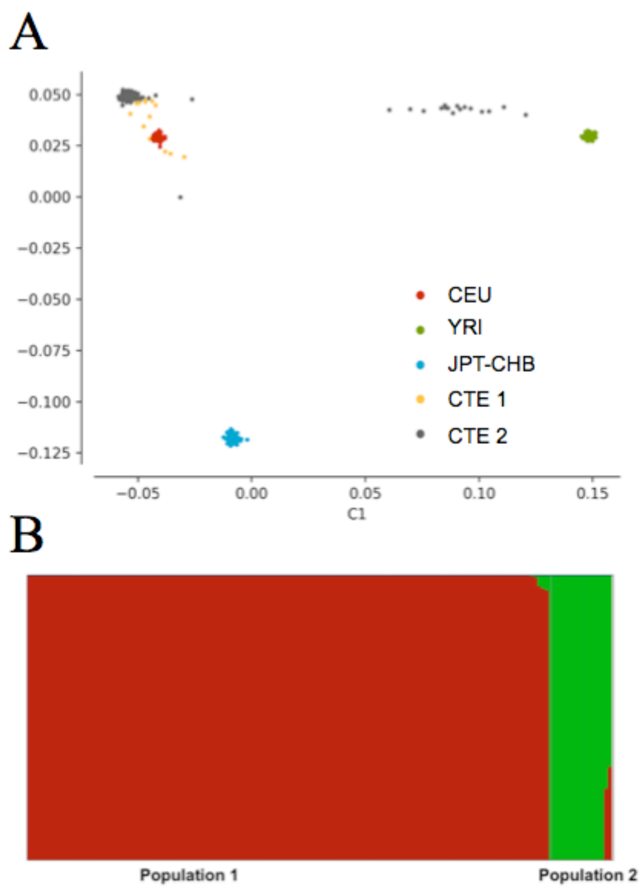

Fig. 1 Determination of population structure. **a** Principle component analysis (pyGenClean) assessing the test cohort ancestry (total *n*=151) compared with the 1000 genomes project references, including CEU (red), YRI (green) and JPT-CHB (blue). The majority of subjects formed a two unique cluster of outliers. The first (*n*=135) was in proximity to the CEU reference above (gray) and within one standard deviation (yellow). The second formed a diffuse cluster (gray) that was closer to the YRI (*n*=16). **b** A distruct plot of the population admixture proportions generated by fastSTRUCTURE demonstrating two subpopulations (x axis, red and green), with small amounts of admixture noted for both (y-axis represents SNPs likely indicative of one or other population). *CEU* Northern Europeans, *YRI* Yoruba, *JPT-CHB* Japanese-Han Chinese.

European cluster. A second smaller more diffuse cluster of admixed Africans was also evident (n=16). We also used fastSTRUCTURE, which uses no reference genomes for comparison, and confirmed the two dichotomous groups. Each of these groups were considered in a combined analysis and independently.

MAPT haplotype association analysis

Using this cohort, with extensive ancestry analysis and autopsy confirmation, we performed our association analysis. We focused on controls with exposure history to maximize our power to detect a modifying effect of the *MAPT* H1 risk allele, which was assessed using rs1052553, a variant that is in complete linkage disequilibrium with the H1 haplotype. In this analysis, we failed to detect a significant association (Table 2).

Table 2. ***MAPT*** **haplotype association analysis***

	Total n	n (cases)	age (mean+/-stdev)	n (controls)	age (mean+/-stdev)	*p* **
Combined cohort	151	112	63.2+/-19.2	39	50.2+/-26.2	0.151
Caucasian only	135	99	64+/-18.8	36	49.9+/-26	0.142

* Using rs1052553 as an H1/H2 haplotype

** Fisher's exact test

MAPT CTE quatitative trait analysis

We next performed a quantitative train analysis using CTE stage 0 – IV as a measure of disease severity in the entire cohort (Table 3). We examined 22 SNPs that were within the *MAPT* gene that were contained within on our chip and passed QC metrics. This analysis uncovered on highly significant SNP, rs2258689, that was marginally significant after Benjamini-Hochberg multiple testing correction.

Table 3.*MAPT*SNPs association with CTE stage (quantitative trait analysis)

	Unadjusted		Adjusted for ethnicity	
SNP	***p***	***p* corrected****	***p***	***p* corrected****
rs2258689	0.002	0.054	0.003	0.076
rs11079727	0.024	0.269	0.036	0.210
rs16940742	0.043	0.290	0.016	0.176
rs8079215	0.053	0.290	0.038	0.210
rs2435200	0.090	0.395	0.087	0.312
rs17651507	0.109	0.399	0.057	0.250
rs2435205	0.145	0.454	0.099	0.312
rs242557	0.214	0.564	0.258	0.487
rs1467967	0.231	0.564	0.212	0.487
rs8078967	0.321	0.566	0.276	0.487
rs1052553	0.343	0.566	0.266	0.487
rs17650901	0.352	0.566	0.255	0.487
rs2471738	0.372	0.566	0.399	0.549
rs4792893	0.383	0.566	0.478	0.619
rs1052551	0.386	0.566	0.288	0.487
rs17651549	0.462	0.635	0.352	0.549
rs17652121	0.515	0.666	0.388	0.549
rs1052587	0.652	0.797	0.520	0.635
rs754593	0.748	0.866	0.831	0.937
rs7521	0.833	0.917	0.937	0.937
rs11867549	0.898	0.941	0.927	0.937
rs2435207	0.946	0.946	0.932	0.937

* Benjamini-Hochberg

CTE genome-wide association study (GWAS).

We used our genotyping data to perform a pilot GWAS using our combined cohort (*n*=151). The five highest ranking SNPs when performing a case-control analysis, are listed (Fig. 2). The highest ranking SNP, rs2978140, was found to be highly significant even after Benjamini-Hochberg correction for multiple testing (*p*=0.018). It is located in the non-coding region on Chr 8q22.1. This SNP railed to reach significance when the population was added as a covariate (not shown).

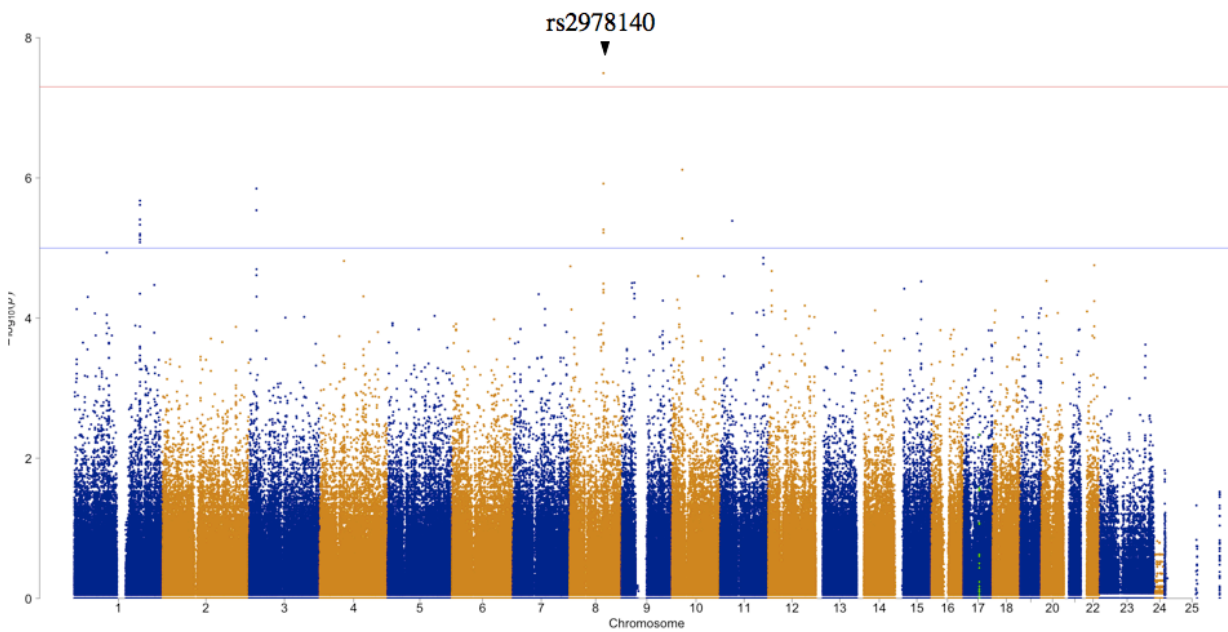

Fig. 2 Manhattan plot of CTE significant SNPs. P-values computed using only CTE stages II-IV as cases. Plot generated using qqman R plugin. Blue/orange colors separate chromosomes. Green dots indicate *MAPT* variants, neither of which is significant. Highest scoring SNP is rs2978140 (uncorrected p-val 3.196e-08, corrected p-val=0.018)

Finally, we performed a genome-wide analysis using CTE stage as a quantitative trait (Table 4). Five SNPs were found to be significantly associated with the stage of disease – a significance that remained even when the population designation and the haplotype were considered (Table 7).

Table 4. Pilot genome-wide association study in CTE

		*p**		
	Nearest gene	Unadjusted	Haplotype adjusted	population adjusted
rs7853409	GRIN1	5.33E-08	3.75E-08	6.64E-08
rs10132438	PACS2	1.80E-07	2.31E-07	1.05E-07
rs1292077	N/A	6.49E-06	5.19E-06	2.47E-05
rs38653	N/A	0.0002688	0.0001148	0.0002977
rs2960060	PCTP	0.004094	0.004257	0.01665

* Benjamini-Hochberg correction

What opportunities for training and professional development has the project provided?

Nothing to report (this is not a training or professional development grant)

How were the results disseminated to communities of interest?

Preliminary genetic findings at the American Academy of Neurology meeting in April, 2017 (Boston). The additional *MAPT* haplotype findings will be presented at the Alzheimer's Association International Conference or the American Association of Neuropathologists meeting.

What do you plan to do during the next reporting period to accomplish the goals?

In this upcoming final year of the award, we plan to:

1. Complete the biochemical assessments of tau isoforms
2. Complete the immunohistochemical studies
3. Publish the *MAPT* association analyses

IMPACT:

*Nothing to report**

CHANGES/PROBLEMS:

There are no additional changes or problems to report.

PRODUCTS:

*Nothing to report**

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*John Crary, MD-PhD*
Project Role:	*Principal investigator*
Researcher Identifier (e.g. ORCID ID):	JC2892 (era commons)
Nearest person month worked:	*1.2 (10% effort)*
Contribution to Project:	*Dr. Crary is overseeing the project.*
Funding Support:	*No additional support*

Name:	*Ann McKee, MD*
Project Role:	*Co-principal investigator*
Researcher Identifier (e.g. ORCID ID):	acmckee
Nearest person month worked:	*1.2 (10% effort)*
Contribution to Project:	*Dr. McKee is coordinating the collection of tissues, neuropathological data and clinical phenotyping.*
Funding Support:	*No additional support*

Name:	*Jesse Mez, MD*
Project Role:	*Collaborator*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*1.2 (10% effort)*

Contribution to Project:	*Dr. Mez has joined the*
Funding Support:	*Dr. Mez's effort is derived from a grant from the Alzheimer's Association to study the genetics of CTE.*

Name:	*Maxim Signaevski, MD-PhD*
Project Role:	*Post-doctoral*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4 (29% effort)*
Contribution to Project:	*Dr. Signaevski is coordinating the genotyping and histopathological studies at Mount Sinai Medical Center*
Funding Support:	*No additional support*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No.

What other organizations were involved as partners?

None

APPENDICES:

*Nothing to report**